

AMENDMENTS TO THE CLAIMS

Listing of the claims:

Claims 1-19. (Cancelled)

Claim 20. (Currently amended) A process for the preparation of thrombolytic enzyme, named [[as]] Thrombinase₂ having a molecular weight in the range of 31,000 to 32,000 Daltons₂ which comprises:

(i) Culturing the filtrate of cells of *Bacillus sphaericus* serotype H5a 5b in a culture medium consisting of 0.03 to 1.5% of yeast extract, 0.2 to 1.5% peptone, 1 to 1.6% sodium acetate, 0.3 to 0.5% beef extract, 0.2 to 0.5% sodium chloride, 0.5 to 1 % Soya peptone, and 0.68% ammonium sulphate at a pH in the range of 7.2 to 8₂

(ii) Removing the cultured cells by cross flow filtration using 0.22.mu filter,

(iii) Subjecting the cell supernatant thus obtained to two step ultra filtration using 100,000 MW (Molecular Weight) cut off membrane followed by ultra filtration of the filtrate thus obtained, using 10,000 MW cut off membrane,

(iv) Salting out the retentate with ammonium sulphate in a concentration in the range of 20 to 40%,

(v) Subjecting the resulting precipitate to dialysis,

(vi) Re-precipitating the precipitate using ice-cold acetone,

(vii) Reconstituting in buffer,

(viii) Decolorizing by using modified CDR (Cell Debris Remover) treatment by eluting with a buffer containing 0.1 to 0.5 M NaCl[[,]] and then dialyzing[[,]] and lyophilizing₂

(ix) Purifying firstly by ion exchange chromatography followed by gel filtration chromatography₂ and

(x) Dialyzing the fraction showing fibrinolytic activity and lyophilizing to obtain purified Thrombinase having a molecular weight in the range of 31,000 to 32,000 Daltons.

Claim 21. (Previously presented) A process as claimed in claim 20 wherein the buffer used is Tris 0.01 M and the pH is 8.0.

Claim 22. (Previously presented) A process as claimed in claim 20 wherein the amount of ice-cold acetone and crude enzyme used are in the ratio of 1:1 to 1:1.5 (v/v).

Claim 23. (Previously presented) A process as claimed in claim 21 wherein the amount of ice-cold acetone and crude enzyme used are in the ratio of 1:1 to 1:1.5 (v/v).

Claim 24. (Currently amended) Thrombinase having a molecular weight in the range of 31,000 to 32,000 Daltons useful for dissolving blood [[-]] clots.